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Article

# Therapeutic monoclonal antibodies: scFv patents as a marker of a new class of potential biopharmaceuticals

**Manuela Berto Pucca[1,*], Thaís Barboza Bertolini[1], José Elpidio Barbosa[1], Simone Vasconcelos Ribeiro Galina[2], Geciane Silveira Porto[2]**

*[1]Molecular Immunopathology Laboratory, Department of Biochemistry and Immunology, Faculty of Medicine of Ribeirão Preto, University of São Paulo, [2]Department of Business Administration, School of Economics, Business Administration and Accountancy of Ribeirao Preto, University of São Paulo*

Monoclonal antibodies represent the fastest growing class of biopharmaceutical products and have a host of applications in medical research, diagnosis, therapy, and basic science. The production of recombinant monoclonal antibodies has revolutionized the generation of immunoglobulins, and their use represents a strategic breakthrough, affecting the global pharmaceutical market for therapeutic proteins. In the present work, a review of scFv, and the number of related patents, has been carried out. The results show that several countries have scFv patents, most notably the United States, China and United Kingdom. The target of these scFv antibodies was also assessed and the results demonstrate that most are directed toward cancer therapy.

**Uniterms:** Monoclonal antibodies. Recombinant antibodies. scFv. Biopharmaceuticals.

Anticorpos monoclonais representam a classe de maior crescimento em produtos de biofármacos e possuem várias aplicações em pesquisa médica, diagnóstico, terapias e ciência básica. A produção de anticorpos monoclonais recombinantes revolucionou a geração de imunoglobulinas e sua utilização implica em avanço estratégico, afetando o mercado farmacêutico global de proteínas terapêuticas. No presente trabalho, uma revisão sobre scFv e a relação do seu número de patentes foi analisada. Os resultados mostram que vários países apresentam patentes de scFv com destaque para os Estados Unidos, China e Reino Unido. Os alvos desses anticorpos também foram avaliados e as análises revelaram que a maioria é destinado a terapias contra o câncer.

**Unitermos:** Anticorpos monoclonais. Anticorpos recombinantes. scFv. Biofármacos.

## INTRODUCTION

Due to their extreme specificity, monoclonal antibodies (mAbs) have been pivotal for analytical advances in the field of medical research, diagnosis, therapy, and basic science. In 1975, Kohler and Milstein published their seminal malignancies manuscript on hybridoma technology, enabling the production of mouse mAbs. Since then, technical advances have allowed the transition from mouse, via chimeric and humanized, to fully human mAbs (Dubel, 2007; Lonberg, 2005), with a reduction in potentially immunogenic mouse components. In this aspect, the use of immunoglobulins submitted to genetic engineering has now become commonplace and represents a strategic breakthrough, affecting the global biopharmaceutical market for therapeutic proteins. This has led to mAbs achieving marked successes in clinical settings (Reichert *et. al.*, 2005; Waldmann, 2003). Indeed, the US Food and Drug Administration has now approved more than 20 mAbs, and more than 150 other mAbs are currently undergoing clinical trials (Reichert, Dewitz, 2006).

The use of monoclonal antibodies (mAb) in biomedical research has been, and will continue to be, important for the identification of proteins, carbohydrates, and nucleic acids. Their use has led to the elucidation of many molecules that control cell replication and differentiation, advancing our knowledge of the relationship between

***Correspondence:** M. B. Pucca. Molecular Immunopathology Laboratory, Department of Biochemistry and Immunology, Faculty of Medicine of Ribeirão Preto – USP. Av. dos Bandeirantes, 3900 – 14040-900 - Ribeirão Preto – SP – Brazil. E-mail: manupucca@usp.br; manupucca@hotmail.com

molecular structure and function. These advances in basic biologic sciences have improved our understanding of the host response to infectious-disease agents and toxins produced by these agents, response to transplanted organs and tissues, to spontaneously transformed cells (tumors), and to endogenous antigens (involved in autoimmunity). In addition, the exquisite specificity of mAb allows them to be used in humans and animals for disease diagnosis and treatment.

The present work originated from a survey on scFv patents, conducted in order to identify the leading countries in the development of technologies involving scFvs. The results were obtained through the mapping of patent records on scFv, and of the applications of these antibodies in different countries. Another aim of this study was to identify scFvs approved to date for therapy, and those undergoing clinical trials.

## Monoclonal antibodies: evolution and perspectives

Antibodies are glycoproteic molecules originating from B cells, which circulate through blood and lymph. They are responsible for the remarkable ability to detect, locate, recognize, bind to an antigen, inactivate it or facilitate its elimination. Each antibody molecule has a unique structure that allows specificity to its corresponding antigen. All of them possess the same general structure and are also called immunoglobulins (Silverton, 1977).

The first experimental evidence of the use of antibodies was proposed by Emil Adolf Von Behring, who demonstrated the use of immunoglobulins to neutralize the diphtheric protein, triggering a revolution in scientific thinking of the time (Nobelpreis, 2009).

Antibodies represent an important category of proteins, not only for their ability to recognize epitopes with high affinity and specificity, but also for their stability and engineering potential. Despite the potential application of the monoclonal antibodies produced by hybridoma technology, their use is limited by their high toxicity. mAbs are proteins normally produced from cells of mice or rats which, when injected into human patients, eventually generate an immune response against the foreign protein, known as a HAMA response (human antimouse antibodies). HAMA is characterized by the development of antibodies by the host, causing an immune response against the administered antibodies, culminating in their neutralization or in a strong immune reaction. This response was a great disappointment for scientists, because it can cause hypersensitivity reactions, such as anaphylaxis and Serum Sickness. Moreover, these antibodies are only effective during the first administration. In subsequent administration, the immune system will be prepared to destroy them before promoting their function (Schroff *et al*., 1985; Shawler *et al*., 1985). This effect is similar to that observed in the use of heterologous antivenoms. Therefore, these responses of the individual against the administration of heterologous protein currently limit the use of these drugs.

Applications using genetic engineering techniques for genes encoding the variable regions (V) and constant (C) monoclonal antibodies, are considered the latest generation of drugs of recombinant antibodies. The process consists of antibody genetic manipulation to make the structure of amino acids more closely resemble the structure found in human antibodies, thereby reducing adverse reactions and HAMA response in patients. A wide variety of molecules can be obtained by this process, which allows different functions. Latterly, the combination of hybridoma technology, recombinant DNA technology and the *Phage Display* technique has allowed the production of antibodies with desirable specificity and affinity (Rees *et al*., 1994).

The *Phage Display* technique was discovered in 1985 by Smith, who developed libraries of peptides on the surface of phages. It is a widely used and well-established technique for the selection and production of antibodies expressed in libraries (Maynard, Georgiou, 2000). Using this technique, it is possible to mimetize the strategy used by the humoral immune system to produce completely human antibodies or fragment antibodies while waiving the immunization process or the construction of hybridomas (Roque *et al*., 2004).

It has been 34 years since the first generation of monoclonal antibodies from B cells of hybridomas of mice (Kohler, Milstein, 1975) and 23 years since the fist approval by the FDA of the use of monoclonal antibodies as therapy (OKT®3, Johnson&Johnson). As with OKT®3 (muromonab), the first monoclonal antibodies were produced exclusively in mouse cells. However, there was an obstacle regarding the use of this discovery. Because the antibodies were of animal origin – mice –, their use in humans caused the HAMA response.

Therefore, scientists strove for years to substitute parts of murin antibodies (momab) with human components (Figure 1). Depending on the way these antibodies are altered, they can be called chimeric antibodies (ximab) or humanized antibodies (zumab). Currently, some technologies, such as *Phage Display* technology, allow the production of totally human antibodies (umab) (Ballow, 2005).

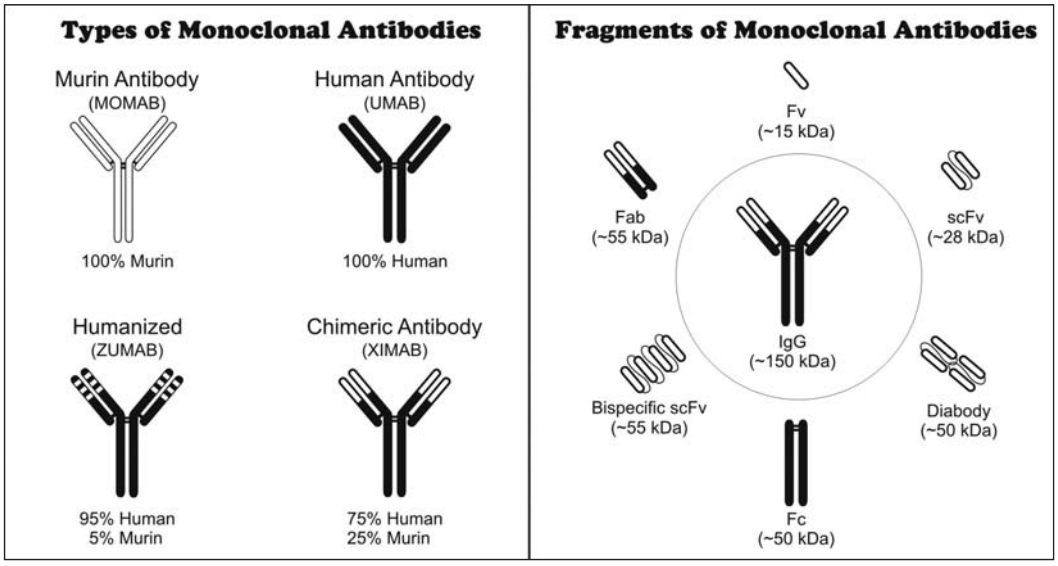


**FIGURE 1** - Types and fragments of monoclonal antibodies.

Research has also been developed using fragments of these antibodies instead of the entire molecule. Because entire antibodies are large molecules (~150 kDa), small fragments derived from IgG can be constructed to stop the antigen-binding activity, such as the minibody fragments Fab, scFv and Fv, or to block the effectors' functions, such as the constant fragment Fc (Figure 1). These small antibodies (~15-55 KDa) can be equipped with radioisotopes or other markers that may be used to locate the target antigen or employed in therapy (Kozak *et al*., 1985).

Among the most studied recombinant antibodies are the Fab (*fragment antigen binding*) and the scFv (*single chain fragment variable*). The single chain fragments (scFv) have been used in both therapy (Chester, Hawkim, 1995) and diagnoses, and have shown advantages over conventional and monoclonal antibodies (Lorimer *et al*., 1996; Turner *et al*., 1997; Wintlow, Filppula, 1991). Such molecules were proven to have rapid blood circulation, since patients treated with scFv presented good localization of the molecules only one hour after their injection (George *et al*., 1996). They also present good penetration in tissues, low immunogenicity in theory, low retention in the kidneys and other non-target organs, better penetration in target tumors, are easily constructed, have low commercial cost in large-scale production (Wintlow, Filppula, 1991) and, moreover, it is possible to restructure them in order to improve their activity and production (Turner *et al*., 1997).

As a result, many institutions worldwide have been carrying out research on scFv antibody fragments, such as Dana Farber Cancer Institution (US), Sloan Kettering Cancer Institution (US), University of Zurich (CN), Northeast Normal University (CN), Nanjin Normal University (CN), Tianjin Medical University (CN), Seoul National University Industry Foundation (KR), Oakland University (US), University Vanderbilt (US), Institut National de la Santé e de la Recherche Médicale (FR), Jandrot-Perrus Martine (FR), University of California (US), Avicore Biotechnology (KR), Unilever PLC (GB), and Unilever NV (NL).

Therefore, assessing scFv patents can be used as a marker of the use of this new class of biopharmaceuticals and also indicate the countries and institutions which have been contributing to the future of antibody therapy in the world.

## Intellectual property

Intellectual property is the general term that expresses the right of inventors or those responsible for any intellectual production, to receive, at least for a certain period of time, reward for their inventions. According to the Worldwide Intellectual Property Organization (WIPO), inventions, literary and artistic work, symbols, names, images, designs and models used in commerce, all constitute intellectual property. Intellectual property comprises two major areas: industrial property (patents, trademarks, industrial design, geographic indications and cultivar protection) and copyright (literary and artistic work, computer programs, Internet domains and intangible culture heritage (Wipo, 2010). Intellectual property makes it possible to transform knowledge, in principle an almost a public asset, into a private asset, and constitutes the link between knowledge and market (Lastres, Ferraz, 1999).

In short, the filing of a scFv patent means that this fragment antibody is a biopharmaceutical, or has the potential to be used as such.

## METHODOLOGICAL ASPECTS

The present work entailed quantitative research on data available from public access patent banks. In order to carry out this study, mapping of the number of worldwide published patents* containing scFv in their title and/or abstract was done. The research was performed using the free-of-charge database *esp@cenet* (http://ep.espacenet.com). The first search on the patent banks occurred in July 2009.

In addition, the registers of EPO - *European Patent Office* (http://www.epoline.org/portal/public/registerplus) and USPTO - *United States Patent and Trademark Office* (http://uspto.gov) were used in order to obtain more details about the patents.

The "Original Document" of each patent was used in the detailed analysis of the requested patents indicated by the mapping. The "Original Document" is a document published by the Intellectual Property (IP) office and contains in-depth information about the record of the analyzed patents.

## RESULTS AND DISCUSSION

The results of the search yielded 315 scFv patents published in the period from January 1996 to July 2009. Figure 2 shows the countries of origin of applicants and the number of patents requested by each country.

Figure 2 shows that the United States has the most requested scFv patents (n=90), followed by China (n=42) and the United Kingdom (n=30). Subsequently, the year of patents requests of the prominent countries were analyzed (the United States, China and the United Kingdom, represented by Figure 3).

Subsequently, the main targets of patent records of the prominent countries were assessed, as shown in Figure 4.

The pharmaceutical industry produces a broad variety of chemical, natural and biotechnological products. This field is essentially based on technological innovation and uses intellectual property in the form of patents in order to own rights to innovations attained through high costs in R&D (research and development). These patents grant market exclusivity and generate high profits (Fardelone, Branchi, 2006).

This work presents a mapping of patents of scFv antibody fragments. The results show that many countries such as the United States, China, the United Kingdom,

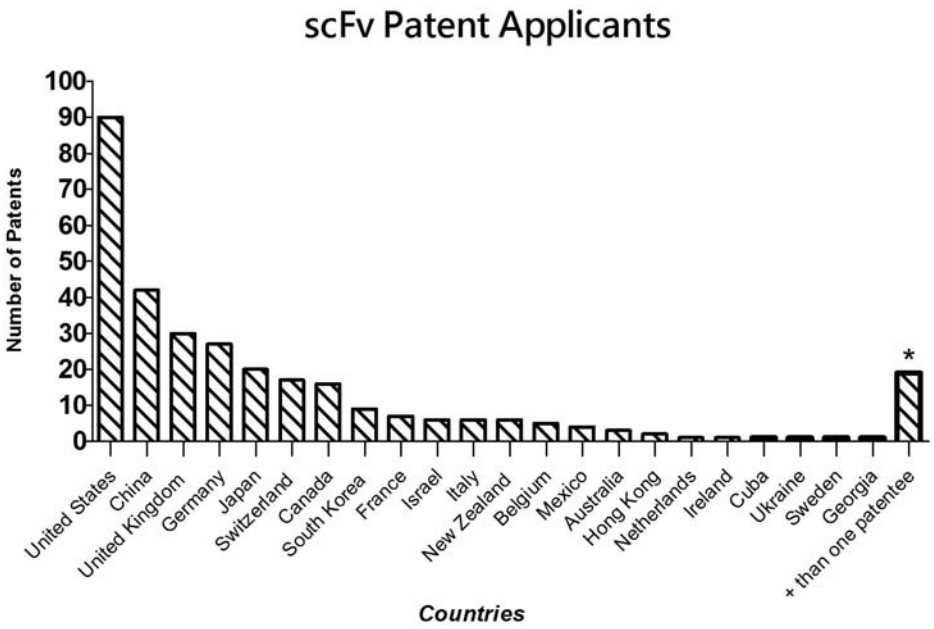


**FIGURE 2** - Number of scFv patents requested by country of origin of applicants (* Patents requested by two countries (1 Japan/United States, 2 Germany/United States, 1 United Kingdom/Austria, 1 United Kingdom /Switzerland, 1 United Kingdom /United States, 1 United States/China, 1 United Kingdom/Germany, 1 Italy/Netherlands, 1 Italy/Slovakia, 1 Switzerland/Austria, 1 Cuba/Netherlands, 2 United Kingdom/Netherlands, 1 Israel/Poland, 1 France/Canada, 1 Sweden/Australia, 1 Russia/Switzerland).

*Published patents are those which have been requested and published after the secrecy period (18 months approximately) and include granted, non-granted and pending patents.

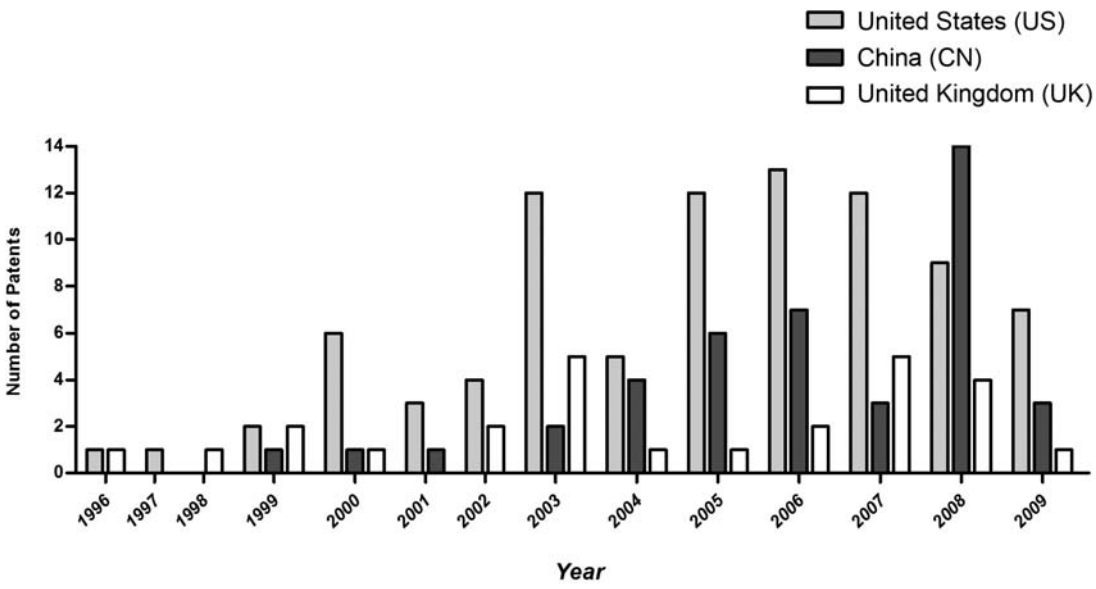


**FIGURE 3** - Distribution of number of scFv patents by year for countries with most patent requests.

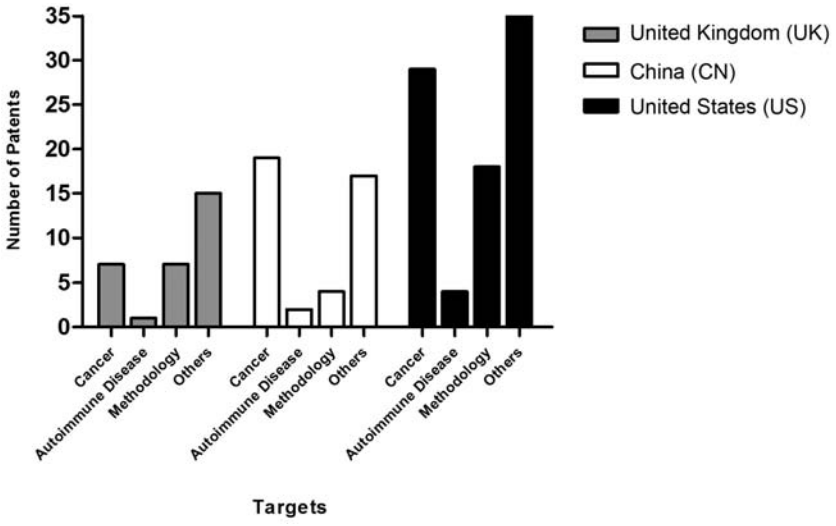


**FIGURE 4 -** Main targets of scFv patents in countries with most requests.

Germany, Japan, Switzerland, Canada, South Korea, France, Israel, Italy, New Zealand, Belgium, Mexico, Australia, Hong Kong, Holland, Ireland, Cuba, Ukraine, Sweden and Georgia hold scFv patent records (Figure 2). The United States, China and the United Kingdom are the prominent patent-holding countries, in other words, they held the greatest number of published patents in the world. A patent study of these countries was therefore carried out, verifying year of publication, the targets of the antibodies and, finally, the application of the knowledge involving scFv production.

The United States was found to have the most records (Figure 3), with 90 patents containing scFv in their titles and/or abstract. This finding was expected since the country invests heavily in R&D (research and development) directed toward pharmaceuticals and is considered a world power in S&T (science and technology). The majority of pharmaceutical product sales occur essentially in the triad comprising the United States, Japan and Europe, together accounting for 88% of all global commercial transactions. The United States are the main market, representing 45% of the sales in a 643-billion-dollar global market (Radaelli, 2008).

In the United States, several antibodies have been authorized by the FDA and, although no scFvs have been approved to date, many scFvs and subtypes are the focus of research (Gupta *et al*., 2010) or are undergoing clinical testing (Table I).

The technology of scFv antibodies in the United States,

**TABLE I** - scFvs undergoing clinical test

| Indication | Brand Name | Target | Stage | Reference/website |
|---|---|---|---|---|
| Melanoma *(scFv)* | SGN-17 | P97 antigen | Preclinical | http://www.seagen.com/ |
| Breast cancer *(scFv)* | F5 scFv-PEG Immunoliposome | Her2 | Preclinical | *Biotchnol. Prog*. **21**, 221-232 (2005) |
| Ovarian and breast cancer *(diabody)* | C6.5K-A | Her2/Neu | Preclinical | *Cancer Res*. **64**, 6200-6206 (2004) |
| Antiangiogenesis and atherosclerotic plaque imaging *(diabody)* | L19 L19–γ IFN | EDB domain of fibronectin | Preclinical | *Int. J. Cancer* **116**, 304-313 (2005) |
| Colorectal cancer imaging *(diabody)* | T84.66 | CEA | Preclinical | *Protein Eng. Des. Sel*. **17**, 21-27 (2004) |
| B-cell tumors *(bispecific scFv)* | BiTE M103 | CD19 and CD3 | Phase 1 | http://www.micromet.de/ |

based on analysis of the patentees (an inventor or institution, such as companies, universities, research institutes), is equally distributed around the country. Several institutions are involved, such as the Dana Farber Cancer Institution, Sloan Kettering Cancer Institution, Oakland University, University Vanderbilt, Biogen Idec, Amgen, Genencor International, Mccormick Alison and Nox Technologies among others. Sometimes the inventor personally requested the patent in the country, but the majority of the patents were requested by an institution. The United States have recorded scFv patents since 1996 (n=1) and there was a great increase in these patents in 2003 (n=12), as shown in Figure 3.

The study of the targets of scFv antibodies over the years (Figure 4) revealed that most patents recorded in the United States involved therapies against cancer (n=29), followed by production methods of these antibodies (n=18). The others were aimed at treatment of autoimmune diseases, immunomodulation, agricultural and animal husbandry plagues, viruses and bacteria, among other highly heterogeneous targets (n=39). In this sense, the knowledge of scFv antibodies is directed toward different therapies and is distributed among research groups all over the country. The perspective is that recombinant antibody technology is set to further increase in the USA, since this technology is linked to a new class of medications, namely, the biopharmaceuticals.

China was ranked in second position (Figure 3) with 42 patents. This position was predictable, for according to a 2006 report by the OECD (Organization for Economic Cooperation and Development), China was set to surpass Japan in investments in 2009, to attain the second position, after the United States.

According to latest estimates, China will invest about 136 billion dollars this year in R&D, slightly more than Japan (130 billion) but still a lot less than the USA, which is the leader in the area, investing 330 billion dollars a year. Although a few Chinese scFv patents were recorded by the inventors themselves, most were recorded by companies and universities, such as Beijing Abt Genetic Engineering Technology, Chengdu Photon Biotechnology, Institute of Medical Biotechnology, Military Medical University, Nanjing Normal University, Northeast Normal University, Shangai Cp Guojian Pharmaceutical, Shenyang Applied Ecology Institute, Tianjin Medical University, Chugai Pharmaceutical (JP), Britain Hills Biomolecule (UK), Terubion Pharmacy (US), among others. The quantity of patents recorded by a single company or university did not exceed three. In other words, none stood out more than the others. The scFv patents have been published in China since 1999, with a great number of recent records in 2008, as shown in Figure 3. Analyzing the targets of scFv antibodies patented by China (Figure 4), most are directed toward cancer therapy, a small number for treating autoimmune diseases (n=2) while the remaining patents are aimed at other applications (n=17). Therefore, the data regarding Chinese scFv patents indicates that knowledge is being generated equally throughout the country, and there is no single outstanding company or university. Moreover, the target of these monoclonal antibodies is directed mainly toward cancer therapy.

The United Kingdom is made up of England, Scotland, Wales and Northern Ireland although some sources refer to England, Scotland, Wales and Northern Ireland as countries. However, even though these possess a certain degree of autonomy, they are not independent, being ruled over by the British Parliament in London. Although ranked third for scFv patent applications (n=30), as shown in Figure 3, the United Kingdom is considered a worldwide leader in innovation. Among the 30 scFv patents requested by the UK, 10 were recorded by Cambridge Antibody Te-

chnology (CAT) 5 of which were recorded in association with the Medical Research Council (MRC). The other 20 patents were recorded by several different patentees, with no distinction among them. CAT is one of the largest biopharmaceutical companies in the UK and a leader in monoclonal antibody therapy, which explains the high number of scFv patents requested by the group. Founded in 1990, its headquarters is located in Granta Park (Cambridge). Using advanced technologies for the isolation of monoclonal human antibodies, it develops numerous and different antibodies independently, or in collaboration with, other researchers. In 2007, there was an integration of MedImmune, CAT and AstraZeneca´s. This integration is currently called MedImmune (*www.medimmune.com)*. Another interesting fact was the higher number of recorded patents in 2003, 2007 and 2008 (Figure 3). Analysis of the targets of scFv patents recorded throughout the years (Figure 4) showed that a large number of patents involved the process of antibody fragment production or, in other words, were related to methodology (n=7). Seven of these were recorded by Cambridge Antibody Technology (CAT) in association with the Medical Research Council (MRC–www.mrc.ac.uk). The same number of patents (n=7) was also found aimed at the treatment of cancer. The target of the remaining patents (n=16) was diverse. In general, it is possible to conclude that the scFv technology in the United Kingdom is concentrated in England (Cambridge) and that attention should be focused on MedImmune. Also, in the past, the therapeutic target of these antibodies was the development of technology to produce these antibodies, whereas focus has now shifted to cancer therapy.

Based on this data, we can conclude that the published scFv patents in the world are most focused on therapies directed at tumors, whereas a minority is focused on autoimmune diseases, similar to the pattern observed in the 3 countries individually. The reason why these applications are focused on cancer therapy is explained by the increased incidence of these diseases and the great difficulty fighting them.

In years to come, the market will be flooded by these latest generation antibodies. Moreover, the technology of monoclonal antibody humanization is linked to a new class of medications, namely, the biopharmaceuticals. These can be directed toward the treatment of important diseases such as cancer, infections and clinical conditions including sepsis, transplant rejection, AIDS and autoimmune diseases (Hon, 2009). The use of immunoglobulins submitted to genetic engineering has become commonplace and represents a strategic advancement involving the global biopharmaceutical market of therapeutic proteins (Rosen *et al*., 1983).

This study showed an increase in scFv patent records and also revealed the applications of the resultant research. Unfortunately, many studies involving this technology did not require patents, precluding a detailed analysis of the situation worldwide. In Brazil, for example, much promising research using scFv antibodies has been conducted, leading to numerous publications in journals (Caldas *et al*, 2000; Maranhão, Brígido, 2000; Oliveira *et al.,* 2008; Pignatari *et al.*, 2007; Tamarozzi *et al.*, 2006). Nevertheless, no published patent related to this research work was found.

### Perspectives

Single-chain antibodies are highly promising in new therapeutic targets directed toward diseases such as cancer. Their use has grown significantly over the past few years, and with continued research, new biopharmaceuticals products will soon be available for use in clinical medicine.

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